

## Article Addendum

# System output of the MAPK module is spatially regulated

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Signaling via the Raf/MEK/ERK (MAPK) module controls multiple cell functions including proliferation, differentiation and survival. How this single pathway can regulate such diverse cell fates is unknown. Recently, we examined system outputs of the MAPK pathway from different cellular compartments. We observed robust activation of the MAPK cascade from both the plasma membrane and the Golgi. When the MAPK module is localized to plasma membrane nanoclusters corresponding to those occupied by activated H-, N- and K-ras, ERKpp output is digital, with both low and high Raf kinase inputs processed to generate a maximal ERKpp output. In contrast, when the MAPK module is localized to the Golgi, ERKpp output is graded such that Raf kinase input corresponds to ERKpp output. These results clearly demonstrate that different cellular environments available to the MAPK module can fundamentally rewire system output, which in turn may allow this single cascade to direct different cell fate decisions.

Within a single cell, the MAP kinase (MAPK) module directs multiple cellular processes vital for cell growth and survival although much ambiguity remains as to how this single pathway can regulate a myriad of cell fates. The small G-protein Ras is a key modulator of the MAPK pathway, transmitting signals from growth factor activated receptors on the plasma membrane to the cell interior. The primary effector of Ras is Raf kinase that is activated by complex mechanisms which include protein-protein and protein-lipid interactions, plus dephosphorylation and phosphorylation of specific residues.<sup>1-3</sup> Active Raf phosphorylates and activates MEK, which in turn phosphorylates and activates ERK. Activated ERKpp has multiple cytosolic and nuclear substrates.

On the plasma membrane H-Ras, K-Ras and N-Ras assemble into spatially and structurally distinct, dynamic nanoclusters that are governed by isoform specific protein and lipid interactions as well as

the activation state of the Ras molecule.<sup>4-8</sup> Ras-GTP nanoclusters are the sites of Raf recruitment to the plasma membrane. Moreover, K-ras nanoclusters operate as digital switches converting a wide range of Raf kinase inputs into fixed outputs of ERKpp. This digital signal output, when coupled with a linear dose response relationship between EGF agonist concentration and the number of Ras nanoclusters generated on the plasma membrane, delivers high fidelity signal transmission.<sup>9-12</sup> In contrast to the plasma membrane, Ras localization and signaling from internal membrane environments is less well understood. However there are well characterized signaling pathways specific for Golgi Ras activation separate from the Grb2/SOS pathway for Ras activation on the plasma membrane.<sup>13-15</sup> Together these observations suggest that spatial and temporal control of MAPK activation may be one mechanism that allows a single biochemical pathway to mediate a portfolio of cell decisions.

To understand how sub-cellular localization regulates signal output, we targeted Raf-1 proteins to membrane environments that operate as MAPK signaling platforms and measured ERKpp output.<sup>16</sup> Using Ras C-terminal sequences, Raf proteins were targeted to all types of Ras plasma membrane nanocluster. This analysis revealed two classes of Ras nanocluster. The first class corresponds to the three spatially distinct type of nanocluster occupied by GTP-bound H-, N- or K-Ras. This class of nanocluster supported robust activation of the MAPK module. The second class corresponds to nanoclusters occupied by GDP-bound inactive Ras, which did not support activation of the MAPK module. Further analysis revealed that all active Ras nanoclusters were able to process low levels of Raf kinase activity into a maximal ERKpp output,<sup>16</sup> thus all active Ras plasma membrane nanoclusters operate as digital switches with respect to ERKpp activation.

To compare endomembrane and plasma membrane MAPK output we directed Raf to the Golgi.<sup>16</sup> Again there was robust activation of ERKpp. However in striking contrast to plasma membrane localized Raf, ERKpp output from Golgi-localized Raf correlated closely with Raf kinase input.<sup>16</sup> Therefore the system output from the MAPK module scaffolded on the Golgi is graded, indicating that different spatial locations can rewire the MAPK module to create different system outputs. This poses the interesting question of how nanoscale environments dictate signaling properties? One clue is that phosphorylation at S338 was strongly associated with Raf activation from the plasma membrane, but not from the Golgi.<sup>16,17</sup> These results and others suggest a role for spatially constrained kinases and co-activators that generate specificity between different membrane environments.

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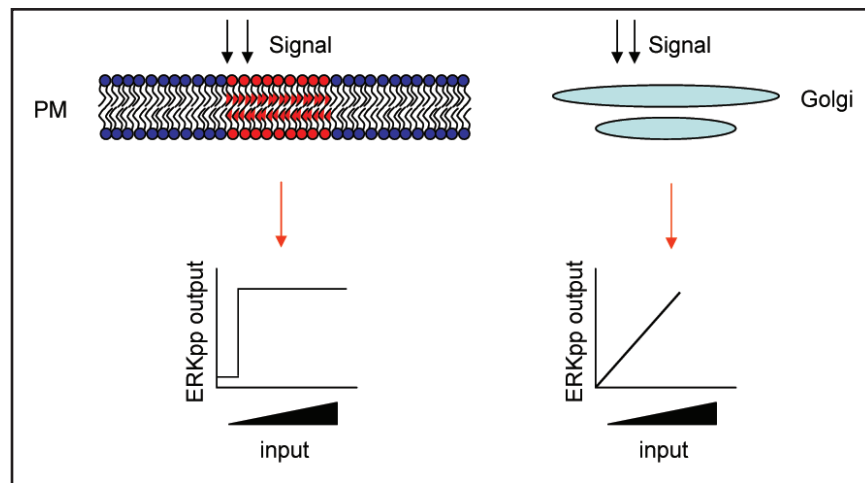


Figure 1. Different cellular environments rewire the MAPK module to generate different system outputs. The MAPK module when scaffolded in plasma membrane nanoclusters functions as a digital switch, converting a range of Raf kinase signal input strengths into maximal ERKpp output. In contrast, the MAPK module when scaffolded on the Golgi functions as an analog processor, delivering a system output that correlates with signal input strength, the fidelity of the response however is low.

By measuring ERKpp output after 2 and 40 minutes of EGF stimulation, we compared endogenous MAPK system output from the plasma membrane and the Golgi in the same cell.<sup>16</sup> Although both the Golgi and plasma membrane ERKpp signal reach the same maximum system output, the Golgi system output peaks at much lower concentrations of EGF input than the plasma membrane. The fidelity of the ERKpp signal responses was quite different: high fidelity at early time points from the plasma membrane, and low fidelity at later time points from the Golgi.<sup>16</sup> Together, our data therefore suggest that the cell receives two quite different levels of ERKpp output from the same EGF input signal. An early accurate read of signal strength generated from the plasma membrane, and a delayed low threshold, almost memory response, from the Golgi (Fig. 1). How these responses are in turn integrated into a biological response is the subject of ongoing work. In summary we show that different MAPK system outputs are possible from different nanoscale plasma membrane compartments and also from different subcellular compartments. These results illustrate how spatially segregated membrane environments can rewire a common biochemical pathway to generate different system outputs.

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